भारतीयमानक
Indian Standard

IS 17924 : 2022

हर्बल सामग्री में कीटनाशक अवशेष निर्धारण

Determination of Pesticide Residue in Herbal Materials

ICS 11.120.10

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Ayush Sectional Committee, FAD 26

FOREWORD

This Indian Standard was adopted by Bureau of Indian Standards, after the draft finalized by Ayush Sectional Committee had been approved by the Food and Agriculture Division Council.

A pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of vegetable drugs. The item includes substances intended for use as growth regulators, defoliants or desiccants and any substance applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.

Due to the increased use of pesticides, contamination in herbal materials has turn out to be one of the major challenges faced by herbal medicine makers. Careful assessment of pesticide residues is therefore, an important step in safety assessment of herbal materials. With this view, the Ayush Sectional Committee – FAD 26 in the Bureau of Indian Standards (BIS) has taken up the task of formulating Indian Standards for determination of pesticide residues among raw materials used in traditional Indian Systems of medicine for the advantage of researchers, academicians, students, clinical practitioners and drug manufacturers

In the formulation of this standard, significant assistance has been derived from the Ayurvedic Pharmacopoeia of India, Part 1, Vol 6, 2008; Ayurvedic Pharmacopoeia of India, Part 1, Vol 8, 2010; and Ayurvedic Pharmacopoeia of India, Part 2, Vol 4, 2017 published by the Ministry of Ayush, Government of India. Inputs have also been derived from the information available in the public domain in print and electronic media including authoritative books.

In the formulation of this standard due consideration has been given to the provisions of the *Drugs and Cosmetics Act*, 1940 and Rules framed thereunder. However, this standard is subject to the restrictions imposed under these Rules and Regulations, wherever applicable.

The composition of the committee responsible for the formulation of this standard is given in Annex A.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test, shall be rounded off in accordance with IS 2 : 2022 'Rules for rounding off numerical values (*second revision*)'. The number of significant places retained in the rounded off value shall be the same as that of the specified value in this standard.

Indian Standard

DETERMINATION OF PESTICIDE RESIDUE IN HERBAL MATERIALS

1 SCOPE

This standard prescribes the test methods for determination of pesticide residue in herbal materials used in traditional medicine.

2 REFERENCES

The following standards contain provisions which through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No.	*Title*
1070 : 1992	Reagent grade water — Specification (*third revision*)
11380 : 1985	Method of sampling for the determination of pesticide residues: Part 1 Agricultural and food commodities

3 APPARATUS

3.1 Size Exclusion Chromatography Instrument

The operative conditions suggested below are typical which can be changed provided the standardization is done:

a) Column – Stainless steel column 0.30 m long and 7.8 mm in internal diameter packed with styrene divinylbenzene copolymer (5 µm);

b) Mobile phase – Toluene; and

c) Flow rate – 1 ml/min.

3.2 Gas Chromatography Instrument

The operative conditions suggested below are typical which can be changed provided the standardization is done:

a) Column – Fused silica column 30 m long and 0.32 mm in internal diameter the internal wall of which is covered with a layer 0.25 µm thick of poly (dimethyl) siloxane; and

b) Mobile phase – Hydrogen for chromatography as the carrier gas. Other gases such as helium for chromatography or nitrogen for chromatography may also be used provided the chromatography is suitably validated.

3.3 Phosphorus Nitrogen Flame Ionisation Detector or an Atomic Emission Spectrometry Detector

3.4 Electron Capture Detector

3.5 A Device Allowing Direct Cold on Column Injection

3.6 Rotary Evaporator

3.7 Oven

3.8 Mechanical Shaker

3.9 High Speed Blender

3.10 Analytical Balance

3.11 Standard Glassware

NOTE — Clean the apparatus and especially glassware to ensure that they are free from pesticides. For example, soak for at least 16 hours in a solution of phosphate free detergent, rinse with large quantities of distilled water and wash with acetone and hexane or heptane.

4 REAGENTS

4.1 Acetone – AR or Equivalent Grade.

4.2 Water – HPLC Grade.

4.3 Carbophenothion – AR or Equivalent Grade.

4.4 Toluene – AR or Equivalent Grade.

4.5 Hexane – AR or Equivalent Grade.

4.6 Acetonitrile – AR or Equivalent Grade.

4.7 Sodium Chloride – AR or Equivalent Grade.

4.8 Sodium Sulphate – AR or Equivalent Grade.

4.9 Diethyl Ether – AR or Equivalent Grade.

5 SAMPLING

The representative samples for estimating pesticide residues shall be drawn in accordance with IS 11380.

6 QUALITY OF REAGENTS

6.1 Reagents including pure chemicals used shall be of analytical grade.

6.2 Reagent grade water for laboratory use shall be as per IS 1070.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which effect the results of analysis.

7 TESTS FOR PESTICIDES

The following methods to be used depending on the substance being examined, it may be necessary to modify, sometimes extensively, the procedure described hereafter. In any case, it may be necessary to use, in addition, another column with a different polarity or another detection method (mass spectrometry) or a different method (immunochemical methods) to confirm the results obtained.

This procedure is valid only for the analysis of samples of vegetable drugs containing less than 15 percent of water. Samples with a higher content of water may be dried, provided it has been shown that the drying procedure does not affect significantly the pesticide content.

7.1 Extraction

To 10 g of the coarsely powdered substance, add 100 ml of acetone and allow to stand for 20 min. Add 1 ml of a solution containing 1.8 µg/ml of carbophenothionin toluene. Homogenise using a high speed blender for 3 min. Filter and wash the filter cake with two quantities, each of 25 ml, of acetone. Combine the filtrate and the washings and heat using a rotary evaporator at a temperature not exceeding 40 °C until the solvent has almost completely evaporated. To the residue add a few ml of toluene and heat again until the acetone is completely removed. Dissolve the residue in 8 ml of toluene. Filter through a membrane filter (45 µm), rinse the flask and the filter with toluene and dilute to 10.0 ml with the same solvent (solution A).

7.2 Purification

Examine by size exclusion chromatography.

7.3 Performance of the Column

Inject 100 µl of a solution containing 0.5 g/l of methyl red and 0.5 g/l of oracet blue in toluene and proceed with the chromatography. The column is not suitable unless the colour of the eluate changes from orange to blue at an elution volume of about 10.3 ml. If necessary calibrate the column, in toluene, using a solution containing, at a suitable concentration, the insecticide to be analysed with the lowest molecular mass (for example, dichlorvos) and that with the highest molecular mass (for example, deltamethrin). Determine which fraction of the eluate contains both insecticides.

7.4 Purification of the Test Solution

Inject a suitable volume of solution A (100 µl to 500 µl) and proceed with the chromatography. Collect the fraction as determined above (solution B). Organophosphorus insecticides are usually eluted between 8.8 ml and 10.9 ml. Organochlorine and pyrethroid insecticides are usually eluted between 8.5 ml and 10.3 ml.

In a chromatography column, 0.10 m long and 5 mm in internal diameter, introduce a piece of defatted cotton and 0.5 g of silica gel treated as follows:

Heat silica gel for chromatography in an oven at 150 °C for at least 4 h. Allow to cool and add drop wise a quantity of water corresponding to 1.5 percent of the mass of silica gel used, shake vigorously until agglomerates have disappeared and continue shaking for 2 h using a mechanical shaker. Condition the column using 1.5 ml of hexane. Prepacked columns containing about 0.50 g of a suitable silica gel may also be used provided they are previously validated.

Concentrate solution B in a current of helium for chromatography or oxygen free nitrogen almost to dryness and dilute to a suitable volume with toluene (200 µl to 1 ml according to the volume injected in the preparation of solution B). Transfer quantitatively onto the column and proceed with the chromatography using 1.8 ml of toluene as the mobile phase. Collect the eluate (solution C).

7.5 Extraction (Method-II)

To 25 g substance being examined, add 300 ml of acetonitrile : water (3:1) and homogenise using a high speed blender for 5 min. Filter and wash the filter cake with two quantities, each of 25 ml of acetonitrile water mixture. Transfer filtrate and rinse to a separating funnel.

Add 50 ml of saturated sodium chloride and mix vigorously for 30 seconds. Add 50 ml hexane to the separating funnel and extract. Repeat extraction with hexane for another two times. Collect the hexane layer and pass the combined hexane layer through sodium sulphate. Collect the hexane and evaporate to dryness. Dissolve the residue in 25 ml hexane.

7.6 Florisil Column Clean Up

Use florisil solid phase extraction cartridges. Using bulb pipet transfer 2 ml of the hexane solution containing the pesticide residue in to the florisil cartridge. Elute with 12 ml of 15 percent diethyl ether in hexane. Further elute with 12 ml of 50 percent diethyl ether in hexane. Collect the elutes separately and evaporate and dry using rotary evaporator. Dissolve in 0.2 ml of n-hexane containing 10 ng/ml of carbophenothion and sonicate.

8 QUANTITATIVE ANALYSIS

8.1 Organophosphorus Insecticides

Examine by gas chromatography, using carbophenothion as internal standard. It may be necessary to use a second internal standard to identify possible interference with the peak corresponding to carbophenothion.

8.1.1 *Test Solution*

Concentrate solution B in a current of helium for chromatography almost to dryness and dilute to 100 µl with toluene.

8.1.2 *Reference Solution*

Prepare at least three solutions in toluene containing the insecticides to be determined and carbophenothion at concentrations suitable for plotting a calibration curve.

8.1.3 *Procedure*

Maintaining the temperature of the column at 80 °C for 1 min, then raising it at a rate of 30 °C/min to 150 °C, maintaining at 150 °C for 3 min, then raising the temperature at a rate of 4 °C/min to 280 °C and maintaining at this temperature for 1 min and maintaining the temperature of the injector port at 250 °C and that of the detector at 275 °C. Inject the chosen volume of each solution. When the chromatograms are recorded in the prescribed conditions, the relative retention times are approximately those listed in Table 1. Calculate the content of each insecticide from the peak areas and the concentrations of the solutions.

Table 1 Relative Retention Time of Pesticides
(*Clause* 8.1.3)

Sl No. (1)	Substance (2)	Relative retention times (3)
i)	Dichlorvos	0.20
ii)	Fonofos	0.50
iii)	Diazinon	0.52
iv)	Parathion-methyl	0.59
v)	Chlorpyrifos-methyl	0.60
vi)	Pirimiphos-methyl	0.66
vii)	Malathion	0.67
viii)	Parathion	0.69
ix)	Chlorpyrifos	0.70
x)	Methidathion	0.78
xi)	Ethion	0.96
xii)	Carbophenothion	1.00
xiii)	Azinphos-methyl	1.17
xiv)	Phosalon	1.18

8.2 Organochlorine and Pyrethroid Insecticides

Examine by gas chromatography, using carbophenothion as the internal standard. It may be necessary to use a second internal standard to identify possible interference with the peak corresponding to carbophenothion.

8.2.1 *Test Solution*

Concentrate solution C in a current of helium for chromatography or oxygen free nitrogen almost to dryness and dilute to 500 µl with toluene.

8.2.2 *Reference Solution*

Prepare at least three solutions in toluene containing the insecticides to be determined and carbophenothion at concentrations suitable for plotting a calibration curve.

8.2.3 *Procedure*

Maintaining the temperature of the column at 80 °C for 1 min, then raising it at a rate of 30 °C/min to 150 °C, maintaining at 150 °C for 3 min, then raising the temperature at a rate of 4 °C/min to 280 °C and maintaining at this temperature for 1 min and maintaining the temperature of the injector port at 250 °C and that of the detector at 275 °C. Inject the chosen volume of each solution. When the chromatograms are recorded in the prescribed conditions, the relative retention times are approximately those listed in Table 2. Calculate the content of each insecticide from the peak areas and the concentrations of the solutions.

Table 2 Relative Retention Time of Insecticides

(*Clause* 8.2.3)

Sl No. (1)	**Substance** (2)	**Relative retention times** (3)
i)	α-Hexachlorocyclohexane	0.44
ii)	Hexachlorobenzene	0.45
iii)	β-Hexachlorocyclohexane	0.49
iv)	Lindane	0.49
v)	δ-Hexachlorocyclohexane	0.54
vi)	ε-Hexachlorocyclohexane	0.56
vii)	Heptachlor	0.61
viii)	Aldrin	0.68
ix)	cis-Heptachlor-epoxide	0.76
x)	o,p-‘DDE	0.81
xi)	α-Endosulfan	0.82
xii)	Dieldrin	0.87
xiii)	p,p-‘DDE	0.87
xiv)	o,p-‘DDD	0.89
xv)	Endrin	0.91
xvi)	β-Endosulfan	0.92
xvii)	o,p-‘DDT	0.95
xviii)	Carbophenothion	1.00
xix)	p,p-‘DDT	1.02
xx)	cis-Permethrin	1.29
xxi)	trans-Permethrin	1.31
xxii)	Cypermethrin*	1.40
xxiii)	Fenvalerate*	1.47 and 1.49
xxiv)	Deltamethrin	1.54

* *The substance shows several peaks.

9 REPEATABILITY AND REPRODUCIBILITY OF TEST METHODS

9.1 The chosen method, especially the purification steps, are suitable for the combination pesticide residue/substance to be analysed and not susceptible to interference from co-extractives;

9.2 The limits of detection and quantification are measured for each pesticide matrix combination to be analysed;

9.3 Between 70 percent to 110 percent of each pesticide is recovered;

9.4 The repeatability of the method is not less than the values indicated in Table 3;

9.5 The reproducibility of the method is not less than the values indicated in Table 4; and

9.6 The concentration of test and reference solutions and the setting of the apparatus are such that a linear response is obtained from the analytical detector.

Table 3 Limits of Pesticide Residues

(*Clause* 9.4)

Sl No. (1)	**Substance** (2)	**Limit (mg/kg), *Max*** (3)
i)	Alachlor	0.02
ii)	Aldrin and Dieldrin (sum of)	0.05
iii)	Azinphos-methyl	1.0
iv)	Bromopropylate	3.0
v)	Chlordane (sum of cis-, trans - and Oxythlordane)	0.05

Table 3 (*Concluded*)

vi)	Chlorfenvinphos	0.5
vii)	Chlorpyrifos	0.2
viii)	Chlorpyrifos-methyl	0.1
ix)	Cypermethrin (and isomers)	1.0
x)	DDT (sum of p,p'-DDT, o,p'-DDT, p,p-DDE and p,p'-TDE)	1.0
xi)	Deltamethrin	0.5
xii)	Diazinon	0.5
xiii)	Dichlorvos	1.0
xiv)	Dithiocarbamates (as CS2)	2.0
xv)	Endosulfan (sum of isomers and endosulfan sulphate)	3.0
xvi)	Endrin	0.05
xvii)	Ethion	2.0
xviii)	Fenitrothion	0.5
xix)	Fenvalerate	1.5
xx)	Fonofos	0.05
xxi)	Heptachlor (sum of heptachlor and heptachlor epoxide)	0.05
xxii)	Hexachlorobenzene	0.1
xxiii)	Hexachlorocyclohexane isomers (other than γ)	0.3
xxiv)	Lindane (γ-hexachlorocyclohexane)	0.6
xxv)	Malathion	1.0
xxvi)	Methidathion	0.2
xxvii)	Parathion	0.5
xxviii)	Parathion-methyl	0.2
xxix)	Permethrin	1.0
xxx)	Phosalone	0.1
xxxi)	Piperonyl butoxide	3.0
xxxii)	Pirimiphos-methyl	4.0
xxxiii)	Pyrethrins (sum of)	3.0
xxxiv)	Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorophenylsulphide)	1.0

NOTES

1 If any pesticides applied to the herb before or after harvesting should also be tested. The limit should be calculated using the following formula:

$$ADI = M/MDD \times 100$$

where

ADI = acceptable daily intake of pesticide as published by Food and Agriculture Organization-World Health Organization (FAO-WHO), in milligrams per Kilogram of body mass;
M = body mass in kilograms (60 kg); and
MDD = Maximum daily dose of the drug, in kilograms.

2 If the drug is intended for the preparation of extracts, tinctures or other pharmaceutical forms whose preparation method modifies the content of pesticides in the finished product, the limits are calculated using the following expression:

$$ADI = M \times E/MDD \times 100$$

Where

E = Extraction factor for the method of preparation, determined experimentally.

Higher limits can also be authorised, in exceptional cases, especially when a plant requires a particular cultivation method or has a metabolism or a structure that gives rise to a higher than normal content of pesticides.

Table 4 Reproducibility of the Method
(*Clause* 9.5)

Sl No. (1)	**Concentration of the pesticide (mg/kg)** (2)	**Repeatability (difference, ± mg/kg)** (3)	**Reproducibility (difference, ± mg/kg)** (4)
i)	0.010	0.005	0.01
ii)	0.100	0.025	0.05
iii)	1.000	0.125	0.25

ANNEX A

(*Foreword*)

COMMITTEE COMPOSITION

Ayush Sectional Committee, FAD 26

Organization	*Representative(s)*
Ministry of Ayush, New Delhi	Dr D. C. Katoch (***Chairperson***)
Association of Manufacturers of Ayurvedic Medicines, New Delhi	Dr V. Sashibhushan Shri Arjun Multani (*Alternate*)
Ayurvedic Drug Manufacturers Association, Mumbai	Dr Nagesh Sandu Shri Nimish Shroff (*Alternate*)
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Member Secretary
Dr Pradeep Kumar Dua
Scientist ‘D’/Joint Director
(Food And Agriculture Department), Bis

Panel for Herbal materials and related subjects, FAD 26/Panel 1

Organization	*Representative(s)*
Pharmacopoeial Commission of Indian Medicine and Homoeopathy, Ghaziabad	Dr G. V. R. Joseph (***Convener***)
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